MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 © 2016. Mycotaxon, Ltd.

July–September 2016—Volume 131, pp. 663–670
http://dx.doi.org/10.5248/131.663

Gymnopus ramulicola sp. nov., a pinkish species from southern China

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Abstract—*Gymnopus ramulicola*, a new species of *G.* subsect. *Vestipedes*, is described from China. It is characterized by a pale red to pinkish white pileus, adnate to subfree lamellae, a (sub)insititious stipe with white mycelium at an enlarged base, a simple pileipellis cutis with occasional weakly diverticulate elements, abundant clamp connections, and habitat on twigs or sticks of broadleaved trees. The new species is compared with similar species in its subsection, and its phylogenetic position is inferred by a maximum likelihood tree based on ITS sequences.

Key words—*Omphalotaceae*, phylogeny, taxonomy

Introduction

During several macrofungal field surveys in southern China, three interesting gymnopoid collections were obtained from Hainan and Jiangxi provinces. Morphological examination and internal transcribed spacer (ITS) sequence analysis indicated that the specimens represented *Gymnopus* (Pers.) Roussel (*Omphalotaceae*, *Agaricales*).

Although 329 *Gymnopus* names have been published, only 204 are currently accepted in the genus (http://www.indexfungorum.org/Names/Names.asp — accessed Oct 9. 2015). China is very rich in fungal diversity, but only 17 species of *Gymnopus* have been reported in the country (Li et al. 2006, Bau et al. 2007, Wu et al. 2009, Deng et al. 2011, Mešić et al. 2011, Zhang & Liu. 2011, Zhang et al. 2012, Li et al. 2012); there are probably many more taxa to be discovered.

Morphological examination of the gymnopoid collections supports placement in *Gymnopus* subsect. *Vestipedes*, which is characterized by small to medium-sized basidiomata, a membranaceous or thin-fleshed pileus, a non-grooved and rootless stipe, a sterile lamella edge with well-differentiated cheilocystidia, and a pileipellis usually with some weakly to distinctly coralloid or diverticulate terminal elements (Antonín & Noordeloos 1997). Most members of this subsection were formerly placed in *Collybia* sect. *Vestipedes* (Kühner 1980, Halling 1983, Jansen 1991), but they were transferred to *Gymnopus* subsect. *Vestipedes* after *Collybia* sensu lato was divided into *Collybia* sensu stricto, *Gymnopus*, and *Rhodocollybia* (Antonín & Noordeloos 1997). After comparison with other species in *G.* subsect. *Vestipedes*, our Chinese taxon was determined to represent a new species, described here as *G. ramulicola*.

Material & methods

Morphological studies

The description of the new species is based on specimens and field photos. Macroscopic features were recorded in the field. Colour codes in the macroscopic description follow Kornerup & Wanscher (1978). Microscopic features were observed from dried material after being sectioned and mounted in 5% potassium hydroxide (KOH) or 1% Congo Red under an Olympus BX51 light microscope (Tokyo). The notation (a–)b–c(–d) is used to present dimensions where the range b–c represents over 90% of the measured values, and both 'a' and 'd' are the extreme values; "n" refers to the number of measured basidiospores, "Q" refers to the length/width ratio of basidiospores, and Q_m refers to the mean ratio. Holotype and paratypes are deposited in the Fungal Herbarium, Guangdong Institute of Microbiology, Guangzhou, China (GDGM).

Molecular procedures and phylogenetic analyses

Genomic DNA was extracted from dried specimens with the Sangon Fungus Genomic DNA Extraction kit (Sangon Biotech Co., Shanghai) according to manufacturer's instructions. The primers ITS1 and ITS4 (White et al. 1990, Gardes & Bruns 1993) were used to amplify nuclear ribosomal internal transcribed spacer region, containing the ITS1, 5.8S and ITS2 regions. Dideoxy sequencing was performed by Beijing Genomic Institute (Guangzhou, China) using the ABI 3730xl DNA sequencer (Applied Biosystems, Foster City, CA, USA). ITS sequences from 22 species taken from GenBank were used to construct a maximum likelihood tree after these sequences were edited and aligned by MEGA 6.0. Relative robustness of individual branches was estimated by bootstrapping 1000 replications.

Results

Molecular results

The phylogenetic analysis contains 25 sequences, among which 22 were downloaded from GenBank. Sequences JN714927 (*Marasmius* type species

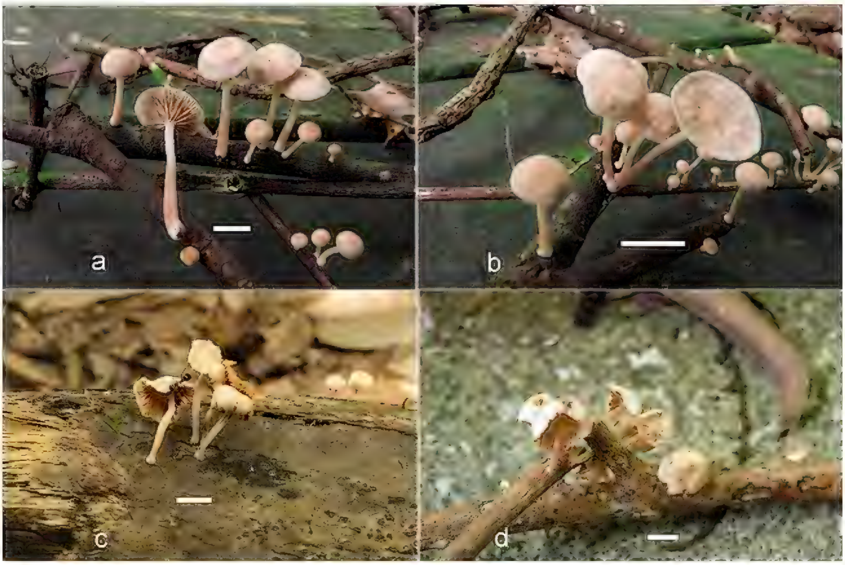

Fig. 1. *Gymnopus ramulicola* basidiomata.
a, b. holotype, GDGM 43884; c. GDGM 44256; d. GDGM 50060. Scale bars = 10 mm.

M. rotula (Scop.) Fr.) and JN021008 (*Collybia* type species *C. tuberosa* (Bull.) P. Kumm.) were selected as outgroup. Maximum likelihood analysis of ITS sequences representing *Gymnopus* sections *Gymnopus*, *Vestipedes*, *Laevipedes*, and *Androsacei* placed our three Chinese sequences in a single lineage and supported our new species as a member of *G.* sect. *Vestipedes*.

Taxonomy

Gymnopus ramulicola T.H. Li & S.F. Deng **sp. nov.** Figs 1, 2

MycoBank MB 815128

Differs from *Gymnopus melanopus* by its pale red to pinkish white pileus, shorter and white to greyish red or dull red stipe, smaller basidiospores, and smaller cheilocystidia; and from *G. confluens* by its smaller pileus, distant lamellae, shorter stipe, and its gregarious habit on fallen twigs.

Type: China, Hainan Province: Ledong County, Jianfengling National Forest Park, 18°43′36″N 108°53′56″E, alt. 787 m asl, 29 June 2013, leg. Wang CQ, Zhou SH (**Holotype**, GDGM 43884; GenBank KU057798).

Etymology: epithet *ramulicola* indicates that the new species is growing on fallen twigs.

Pileus 5–25 mm broad, hemispherical to convex when young, expanding to applanate with a slightly uplifted margin, pale red (7A3, 8A3, 9A3) to pastel red (7A4, 8A4) at disc and reddish or pinkish white (9A2, 10A2) at margin when

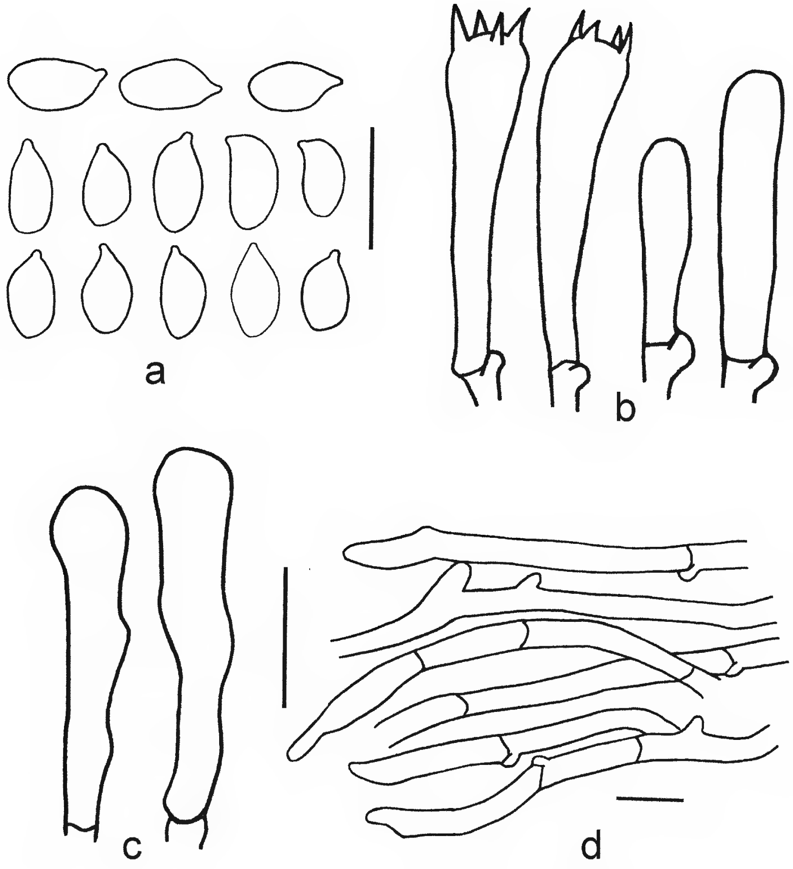

Fig. 2. *Gymnopus ramulicola*. (holotype, GDGM43884).
a. basidiospores; b. basidia and basidioles; c. cheilocystidia ; d. pileipellis. Scale bars = 10 µm.

young, sometimes greyish orange (6B3–4), greyish red (7B3–4) or dull red (8B3, 8C3), usually paler when mature, becoming orange white or pale orange (5A2–3, 6A2–3) to yellowish white or pale yellow (3A2–3, 4A2–3) when old, dry, with inconspicuous radial stripes or grooves and fine white tomentum. Lamellae adnate to sub-free, narrow, distant, with 9–12 complete lamellae and 1–4 lamellulae of different lengths between the two complete lamellae, concolourous with pileus to somewhat dull red or greyish red (8B4 to 9B5, 8C4–5). Stipe central, 12–23 × 2–3 mm, cylindrical, hollow, often slightly inflated at the base, (sub)insititious, white at apex, becoming greyish red (7B3–4) or dull red (8B3, 8C3) downwards, dry, covered with fine white tomentum, and with white mycelium at base. Context thin, whitish to similar to pileus colour. ODOUR and TASTE not distinctive.

BASIDIOSPORES (n = 30) (6.3–)6.6–8.4(–8.8) × (3.2–)3.5–4.5 µm, Q = 1.6–2.4, Q_m = 1.94, ellipsoid to oblong, smooth, thin walled, hyaline. BASIDIA 23–27 (–30) × 3.8–5.5 µm, clavate to cylindrical, inflated at apex, thin-walled, with (2–)4 sterigmata, clamped. BASIDIOLES 16–25 × 3.0–4.5 µm, cylindrical, clamped. CHEILOCYSTIDIA 23–27(–30) × 3–6 µm, indistinct, irregularly clavate to cylindrical, inflated at apex, hyaline, thin-walled. PLEUROCYSTIDIA absent. PILEIPELLIS a cutis of radially arranged cylindrical hyphae which are 4–9 µm in diam., smooth, septate, thin-walled, and weakly to distinctly diverticulate. CLAMP CONNECTIONS abundant and present in all tissues.

HABITAT—gregarious on fallen twigs in tropical or subtropical broadleaf forests.

ADDITIONAL SPECIMENS EXAMINED: CHINA, HAINAN PROVINCE: Changjiang County, Bawangling National Forest Park, 19°07′25″N 109°05′35″E, alt. 657 m asl, 6 June 2013, leg. Wang CQ, Zhou SH (GDGM 44256; GenBank KU321529); JIANGXI PROVINCE: Ganzhou City, Chongyiyang National Forest Park, 55°38′99″N 114°19′10″E, alt. 374 m asl, 2 August 2013, leg. Zhang M, Xu J, Zhou SH (GDGM 50060; GenBank KU321530).

Discussion

Among the species with ITS sequences in the phylogenetic tree (FIG. 3), *Gymnopus ramulicola* is closest to *G. melanopus* A.W. Wilson et al., which differs by its dark brown to greyish orange or beige pileus, its longer (21–55 mm) brown to black stipe, its larger basidiospores (7.2–10.4 × 3.2–5.6 µm), and its much larger cheilocystidia (32–56 × 9.5–16.5 µm; Wilson et al. 2004). The second closest taxon in the tree is *G. nonnullus* var. *attenuatus* (Corner) A.W. Wilson et al., which differs by its dark chocolate brown to grayish brown pileus, its larger basidiospores (7.2–9.6 × 3.2–4.8 µm), and its much larger cheilocystidia (37.5–60 × 5.5–7 µm; Wilson et al. 2004).

Morphologically, *G. confluens* (Pers.) Antonín et al., *G. huijsmanii* Antonin & Noordel., *G. inodorus* (Pat.) Antonín & Noordel., *G. moseri* Antonín & Noordel., and *G. putillus* (Fr.) Antonín et al. (all in *G.* subsect. *Vestipedes;* Antonín & Noordeloos 1997) somewhat resemble *G. ramulicola*. However, *G. confluens* has a bigger (10–65 mm), pale brown, yellow-brown or pale grey-brown pileus, more crowded lamellae, and a longer, distinctly non-insititious stipe (25–90 × 1.5–4 mm; Halling 1983, Antonín & Noordeloos 1997); *G. huijsmanii* has incarnate to violaceous brown stipe, larger basidiospores (6.5–11.5 × 3.5–5.5 µm; Antonín & Noordeloos 1997); *G. inodorus* has more

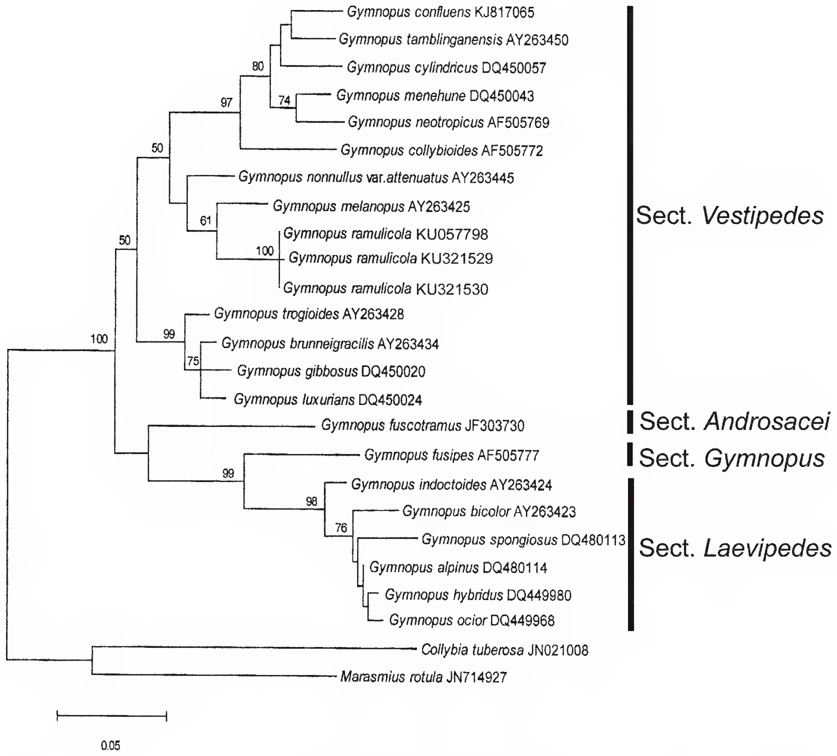
Gymnopus confluens KJ817065
Gymnopus tamblinganensis AY263450
Gymnopus cylindricus DQ450057
Gymnopus menehune DQ450043
Gymnopus neotropicus AF505769
Gymnopus collybioides AF505772
Gymnopus nonnullus var.attenuatus AY263445
Gymnopus melanopus AY263425
Gymnopus ramulicola KU057798
Gymnopus ramulicola KU321529
Gymnopus ramulicola KU321530
Gymnopus trogioides AY263428
Gymnopus brunneigracilis AY263434
Gymnopus gibbosus DQ450020
Gymnopus luxurians DQ450024
Gymnopus fuscotramus JF303730
Gymnopus fusipes AF505777
Gymnopus indoctoides AY263424
Gymnopus bicolor AY263423
Gymnopus spongiosus DQ480113
Gymnopus alpinus DQ480114
Gymnopus hybridus DQ449980
Gymnopus ocior DQ449968
Collybia tuberosa JN021008
Marasmius rotula JN714927
80
74
97
50
61
100
50
100
99
75
99
98
76
Sect. Vestipedes
Sect. Androsacei
Sect. Gymnopus
Sect. Laevipedes
0.05

FIG. 3. Phylogenetic tree based on the ITS rDNA region (ITS1, 5.8S rDNA and ITS2), maximum likelihood tree after sequences being edited and aligned by MEGA 6.0. Relative robustness of individual branches was estimated by bootstrapping 1000 replications. Bootstrap values (>50%) are shown above individual nodes.

crowded lamellae and strongly inflated cheilocystidia that typically have finger-like apical excrescencies (Antonín & Noordeloos 1997); *G. moseri* has a brown to red-brown stipe and larger basidiospores (8.5–11.0 × 4.0–5.0 µm; Antonín & Noordeloos 1997); and *G putillus* has more crowded lamellae, a brown or greyish brown, white to greyish pruinose stipe, and no cheilocystidia (Antonín & Noordeloos 1997);

Among the other species in *Gymnopus* sect. *Vestipedes* with clamp connections, thin-fleshed pileus, and a non-grooved and non-insititious stipe, *G. allegreti* (De Seynes) A.W. Wilson et al. is distinguished by a light brown to pale yellowish brown pileus, a longer stipe with coarse rhizomorphs, and larger cheilocystidia (33–46.5 × 6.5–10 µm; Wilson et al. 2004); *G. brunneigracilis* (Corner) A.W. Wilson et al. has a rusty reddish brown to light brown pileus and larger basidiospores (8–13.6 × 4.0–6.1 µm; Wilson et al. 2004); *G. collybioides* (Speg.) Desjardin et al. has smaller basidiospores (5.6–7.2 × 2.4–4.0 µm) and basidia (16–18 × 4–5 µm; Mata & Petersen 2003); *G. cylindricus* J.L. Mata has crowded lamellae and longer basidiospores (8.8–13.6 × 3.2–4.8 µm; Mata et al. 2004); *G. indoctus* (Corner) A.W. Wilson et al. has a brownish beige or creamy beige to white pileus, a longer, fibrillose stipe with rhizoids at the base, smaller basidia and somewhat larger cheilocystidia (26.5–35 × 9–12 µm; Wilson et al. 2004); *G. johnstonii* (Murrill) A.W. Wilson et al. has a dark brownish grey to brown pileus, more crowded lamellae, smaller basidia (12–17.5 × 5.5–10.5 µm), and cheilocystidia bifid or with a few lobes (Wilson et al. 2004); *G. luxurians* (Peck) Murrill has a larger, reddish brown to light brown pileus, more crowded lamellae, a stipe with rhizoids, and somewhat diverticulate cheilocystidia (Halling 1983); *G. menehune* Desjardin et al. has a reddish brown to dark brown or brown young pileus, close to crowded lamellulae, larger basidiospores (7.5–9.5 × 3.5–4.2 µm), and lobed and diverticulate cheilocystidia (16–42 × 3.8–10.0 µm; Desjardin et al. 1999); and *G. virescens* A.W. Wilson et al. has a dark reddish brown to light brown or light grey pileus, crowded lamellulae, a few coarse rhizomorphs at the base of stipe, larger basidiospores (7–10 × 3–6 µm), and a lamellar edge lacking cheilocystidia (Wilson et al. 2004).

Acknowledgments

Thanks are given to Shi-Hao Zhou, Ming Zhang and Jiang Xu for their help in collecting the specimens. We are grateful to Dr. Vladimír Antonín (Moravian Museum, Department of Botany, Brno, Czech Republic) and Dr. Armin Mešić (Ruđer Bošković Institute, Zagreb, Croatia) for their pre-submission reviews. This work was supported by the National Natural Science Foundation of China (Nos. 31260011, 31570020), the Ministry of Science and Technology of the People's Republic of China (Nos. 2014CB460613, 2013FY111200) and the Science and Technology Planning Project of Guangzhou (No. 201504291620324).

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